prove important in revealing the roles of aggrecanase versus MMP aggrecanolysis in the IGD of aggrecan at different stages of human OA.

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INCREASED SYNOVIAL FLUID AND DECREASED SERUM BONE ALKALINE PHOSPHATASE CONCENTRATIONS ASSOCIATED WITH EQUINE OSTEOCHONDRAL FRAGMENTATION

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Aim of study: To determine the bone alkaline phosphatase (BAP) concentrations in the synovial fluid and serum from horses with osteochondral fragmentation compared to normal, treadmill-exercised horses.
Methods: Synovial fluid was collected from the carpal joints and blood was collected from the jugular vein of 9 mature Thoroughbred horses presented for arthroscopic debridement of osteochondral fragmentation. As a comparable exercise control group, serum and carpal synovial fluid samples were collected from 14 mature Thoroughbred horses after 6 weeks of regular treadmill training. BAP concentrations were measured in the synovial fluid and serum using a commercially available immunoassay (Metra™ BAP, Quidel Corporation, San Diego, CA). Synovial fluid and serum were compared between the osteochondral fragment group of horses and the exercise control group using an unpaired t-test. A value of $P<0.05$ was considered significant.
Results: The synovial fluid BAP concentration was significantly elevated ($P<0.01$) in the horses with osteochondral fragments (mean $\pm$ standard deviation, 21.87 $\pm$ 8.4 U) compared to the exercise control horses (13.32 $\pm$ 6.44 U). However, the serum BAP concentration was significantly lower ($P<0.001$) in the horses with osteochondral fragments (31.45 $\pm$ 8.44 U) compared to the exercise control horses (52.33 $\pm$ 11.64 U).
Conclusions: The elevated concentrations of BAP in the synovial fluid of horses with osteochondral fragments compared to exercise control horses may be due to local production and release of the enzyme from the exposed subchondral bone and inflamed synovium. In addition, delayed clearance of BAP from the joint as a result of joint effusion may also result in greater synovial fluid concentrations. The lower concentrations of BAP in the serum of horses with osteochondral fragmentation compared to exercise control horses may be due to the basic physiologic response of bone to exercise compared to injury. In the uninjured, exercised horse, bone formation (modeling) predominates to adjust to the loads applied during exercise. However, when acute injury to the bone occurs, as with osteochondral fragmentation, the bone may go through a remodeling process. If so, the bone must first go through a resorptive period before the osteoblasts would be recruited to the site to initiate new bone formation. Therefore, due to this relatively delayed response of the osteoblasts, the serum BAP concentrations may be lower in the osteochondral fragmentation horses compared to horses with exercise-induced modeling. Examination of both the synovial fluid and serum BAP concentrations in various types of joint disease is valuable in determining the different local and systemic effects that occur in response to the joint disease.

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EVALUATION OF AGGRECAN FRAGMENTS BY USING TWO COLOR INFRARED IMAGING DETECTION

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The ability to quantitate aggrecan fragments and their neoepitopes in both plasma and synovial fluids is important for the development of biomarkers that will monitor aggrecan degradation in osteoarthritis. While the ultimate goal of these biomarker efforts is the development of high sensitivity, reproducible assays capable of evaluating large number of clinical and preclinical samples, it is essential that these results can be independently validated using different technology to insure the specificity of the immunoassays under development. Accordingly, the present study has focused on the detection of aggrecan fragments by Western blot analysis using two color infrared imaging technology. Initial studies have focused on the detection of a 32 amino acid peptide fragment (32-mer) following aggrecan digestion with matrix metalloprotease 13 and ADAMTS 4/5. A rabbit affinity purified polyclonal with recognition of the carboxy terminus and a mouse monoclonal recognizing the amino terminus of the peptide were used to detect the 32-mer by Western blot analysis following SDS gel electrophoresis of the aggrecan digests. The fluorochrome conjugated detection antibodies (anti rabbit and anti mouse) were chosen based on the 780 and 680 excitation wavelengths of the Odyssey's two diode lasers. This technology insures the specificity of the antibodies used for higher throughput assays by requiring co-staining with both antibodies to the aggrecan peptide of interest and is easily visualized in the same sample lane. The ability to utilize this approach in complex biologic matrices will provide a greater understanding of both neoepitope levels as well as the potential size diversity of the neoepitope containing aggrecan fragments in both clinical and preclinical samples.

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DEVELOPMENT OF COMPETITIVE ELISAS FOR THE DETECTION OF AGGRECANASE CLEAVED FRAGMENTS OF AGGRECAN

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The cleavage of aggrecan by aggrecanases (e.g. ADAMTS-4/5) generates a series of neoepitope exposed fragments which are thought to be potential candidates for Osteoarthritis (OA) associated biomarkers. The ability to detect these neoepitope fragments in patient samples could potentially aid physicians and researchers in providing additional criteria in conjunction with clinical signs for selecting the most appropriate and effective treatments for symptom amelioration or disease modification. In the present study, a series of competitive enzyme-linked immunoabsorbent assays (ELISAs) have been developed to quantitate OA biological samples for the most abundant neoepitope fragments using affinity purified rabbit polyclonal antibodies (poABs) derived following synthetic peptide immunizations. These antibodies were characterized by BIACORE analysis with K_D values for the specific neoepitope sequences between 10^{-10} to 10^{-8}M. In the competitive ELISA format, the assay measures the ability of